Dever, Thomas 2021

Dr. Thomas Dever Oral History

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Dr. Thomas Dever

Behind the Mask

March 12, 2021

GB: Good morning. Today is March 12, 2021, and I have the pleasure of speaking with Dr. Thomas Dever. Dr. Dever is a senior investigator in the Section of Protein Biosynthesis at the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Thank you very much for being with me today.

TD: Oh, it's my pleasure. Thank you.

GB: Yes, so in simple terms can you explain the key elements of your research and the importance of understanding frame shifting?

TD: I guess we should start with: the virus encodes a lot of proteins, and right near the beginning of the virus, it encodes two polyproteins, “poly” meaning many. These are fusion proteins that have a number of smaller sub-unit proteins within them, but they're called orf-1a and orf-1ab. As the name implies, orf-1ab is just an extended version orf-1a. It's this extension that caught our interest, and it's been an interest [to] a number of researchers, and it's not just in SARS but other viruses as well.

What happens is the ribosome (remember ribosomes are what translate and make the proteins), they read the mRNA three nucleotides at a time or a codon. We call three nucleotides a “codon”. The ribosome moves down in groups by three [nucleotides], three, three each time, moving down and putting in a different amino acid, whatever that nucleotide codon designates, but that's how orf-1a is made. Then the ribosome gets to what we call a stop codon, and then the ribosome disengages from the message.

And this is what happens in orf-1ab: the ribosome gets down to a sequence called a shifty sequence or a slippery sequence, and at this position, the ribosome instead of moving by three nucleotides actually only moves by two nucleotides, and now it's going to start reading in a different frame. So instead of going three, three, three, three, now it's going to go three, three, three, two, and then it's going to start reading three by three again, but now it's going to be offset by one nucleotide compared to what it was reading before. And it's now able to extend and make a larger protein, and this is something that the SARS virus and—HIV actually does this as well but in a different context, of course— but this frameshifting is required to make this orf1ab protein.

The [SARS-CoV-2] virus, and most all viruses, want to have a specific ratio of orf-1a to orf-1ab, and so the rate of this frameshifting is going to determine how much orf1a protein is made and then how much orf-1ab protein is made, and the key protein in orf-1ab is the viral RNA polymerase. This is the main enzyme that the virus uses to make more copies of itself, and so it needs to have this frameshifting occur at the right frequency in order to make more of this [polymerase] protein that allows it to make more copies of itself. It's been shown for other viruses that if you just alter this ratio of orf-1a to orf-1ab, the virus has problems replicating and making more copies of itself, and so if we can disrupt the frequency, either increasing the frequency of frameshifting so it makes more polymerase compared to the earlier proteins or if it makes a lot less polymerase compared to the earlier proteins, it can disrupt viral replication. We are interested in…

GB: How do you do that?

TD: There are many different ways. Some people screen for drugs that might alter the ability of the ribosome to shift, but we have decided to study two very specific, should I say, proteins that can impact frameshifting. One is a protein that was just recently characterized about maybe two years ago. A group identified a protein that gets induced by interferons. So, I think you've heard of interferons. They are a part of our antiviral defense mechanism. So, there's a gene product of unknown function; it has a very generic name c19orf66, but it's known to be induced by interferons. There's a lab that claims that when this protein gets overexpressed, it interferes with frameshifting. We are interested in studying if over-expression of that protein interferes with SARS frameshifting. So that's a very straightforward experiment, and we have data that suggests that it does affect frameshifting.

And then another protein that we study is actually what's called a translation factor, and this is something that binds to the ribosome and helps the ribosome synthesize proteins. The translation factor has a modification (or decoration) on it, and when the modification is gone, frameshifting increases about one and a half times. The notion would be, well, maybe an inhibitor of that modification might be of potential therapeutic value, but before we get that far down the road, we first need to characterize what does this modification do and how does it impacting frameshifting?

So those are the basic science type questions that we're studying. My lab is really just trying to figure out how does this frameshifting occur, how do c19orf66 and this other factor, which is called elongation factor two (eEF2), how do they contribute to this frameshifting event in the SARS virus?

GB: Are you comparing one versus the other, like what does better in terms of stopping the virus from replicating or would it be in tandem that you would hope that you would have to do both of those?

TD: We have done some experiments to look at the interplay between the two of them, but actually I think we should take a step back, and I should let you know that we actually aren't studying the virus. So, what's really interesting about this frameshifting event is that we can narrow the frameshifting element down to as few as 40 to 100 nucleotides. We just take a little segment of the virus around 100 nucleotides, and we can put it into some other, what we call, “reporter genes”, and we can just study that tiny little segment. We can show that that [the frameshifting element] alone can promote frameshifting, and so we can study frameshifting just in the context of this little segment. We collaborate with other labs to actually then move our studies into a true viral infection assay. We're actually sending some of our mutant cells to a lab right now who will do the actual infection assays.

GB: At NIH or [with] a collaborator?

TD: An collaborator outside of NIH is going to do this for us.

GB: Yes interesting. So does your technique… it sounds like it would work for possibly other viruses too.

TD: Potentially so. This is actually where you—so c19orf66 has already been published to inhibit frameshifting by HIV and some other viruses, and this eEF2 modification has also been shown to affect frameshifting on HIV. We now clearly have it (the eEF2 modification) affecting SARS frameshifting as well, but then I think it gets into the question of how much does it affect frameshifting? Because the frameshifting elements in HIV and SARS are very different and so maybe that (the modification) changes it (frameshifting) by one and a half fold for one virus, but maybe it changes it by fivefold for another. I'm just making up numbers, but it potentially could have different consequences for different viruses and how sensitive the different viruses are to changes in frameshifting.

GB: Have you been looking at SARS before the pandemic occurred?

TD: No. We had not looked at SARS, but we had looked at HIV’s frameshifting. We actually are studying frameshifting for some cellular genes, so this is not just viruses. Some of the genes in our body actually use frameshifting, and we've been studying some of those as well, and so it was a natural move for our lab to study SARS (frameshifting) in this aspect of it.

GB: When did you all start studying SARS frameshifting, and what was that move like for you like in terms of having to catch up on the particulars?

TD: I guess we probably started in April because the labs closed down in March. They sent out notices if you wanted to work on SARS-CoV-2, and we said, “Well, we might as well work on this,” because we thought that this eEF2 modification is something that not many labs work on, and so we thought we had a unique angle in that way. We started working on it (SARS frameshifting) right away, and because we worked on reporter assays for other viruses and knew only needed to use the small segments of the virus, it was very quick for us to order the segment of DNA that we needed and make the reporter. It was a very seamless transition for us in that way, and we were familiar with, remember, the original SARS virus. I guess [it] would now be called SARS-CoV-1. SARS-CoV-1 has a frameshift site, and people have been studying it for a while. The frameshift element from the original SARS virus, and now SARS-CoV-2 are (nearly) identical – I think there's one nucleotide difference out of 100 nucleotides.

GB: Wow. That's very, very interesting. What have been some of the challenges you have faced with your research, COVID research so far, and have there been any surprise findings? It sounds like there's been great success with this elongation approach.

TD: So, challenge first. Well, just like everyone else the challenge is getting into the lab with the safety precautions that we're using, so you know that slows our pace of our research, but we've actually done pretty well. All in all, we're not complaining because we're happy to be able to continue to do research, though one of the critical features is actually getting collaborators who can do these infection assays. It's not that simple because there's only a certain number of labs in the country that are equipped to handle SARS and the safety precautions, and you can imagine there are so many people with so many great ideas who are contacting this limited group of labs that can do the infection assays. So, to get a lab that can do the assays is a real challenge. We thought about some other strategies. I mean, could we go to a lab that maybe studies a mouse virus that is similar to SARS and might be able to do it do it for us? But it turns out that it's not that simple for what we wanted to do. We really needed to find a lab that could work with SARS-CoV-2 itself, and fortunately now we have just recently found a lab that is able to do the experiments for us, and what's really nice is the collaborator we've identified is someone who's actually interested in protein synthesis as well so it really went quite well.

GB: That's really, really great. This is more of, I guess, a technical question, but do you study all three types of RNA polymerase, or do you focus on one type?

TD: We don't study RNA polymerase; we study more or less… we study the ribosome, and so, we study protein synthesis and not RNA synthesis. I mean our RNA polymerases are required for production of the ribosome, but we don't study ribosome production. We study it (the ribosome) once it's assembled and how it (the ribosome) works to make proteins.

GB: That makes more sense. What do you think are some practical or concrete applications from your basic science research with SARS-CoV-2?

TD: I mean this is very speculative. So, if this c19orf66 protein would be a potential inhibitor of (SARS-CoV-2) frameshifting, then you could imagine: is there a way that we could induce expression of that protein? or provide that protein into cells?, and is (this) a way to disrupt viral replication. That would be a potential application. The modification on eEF2: if we could [find] a small molecule that might prevent its modification, [this] potentially could prove to be of benefit. These would be potential therapeutics that could block replication, but admittedly this is a long-term process. What we're working on is not something that's going to be immediate. It's not going to provide an immediate response or an immediate gratification in terms of identifying something important for the virus. But now that we know that these viruses are around, they're going to be around forever, and there's going to be more coronaviruses, the insights that we obtain might not be beneficial in this current pandemic but might be of benefit in the future.

GB: Definitely. Can you talk a little bit about…I was just wondering how you go about doing the technical aspects of your research?

TD: What we do is we make these reporters, and so we have some very sensitive reporters called luciferase. They're actually based on the enzyme that fireflies make for their lighting, and so we can make what we call a dual luciferase reporter. We have one luciferase gene and then we have another (second) luciferase gene downstream of it in the mRNA, and they're (translation frames are) offset by one nucleotide. So, after the ribosome translates the first luciferase, production of the second luciferase is going to require a shift of reading frame. We put in the virus frameshifting element after the first luciferase gene and before the other luciferase gene called Renilla luciferase. So both luciferase genes will be translated if the viral element promotes frameshifting. We can look at the ratio of these two (luciferase proteins) to determine how much frameshifting is occurring on this little (viral) sequence that we insert in the middle (between the two luciferase genes) …

GB: Do you have a tracker?

TD: There are assays that will specifically look at two different kinds of luciferase activity, and so we can look at how much of the firefly luciferase is made and how much of the Renilla luciferase is made, quantify those in our assays, and then by their ratio, we know how much frameshifting is occurring. Then we can over-express c19orf66, and that changes the ratio – we see the same amount of the first luciferase (firefly), but less of the second one (Renilla).

GB: Okay.

TD: Or when we block the modification on eEF2, we get more of the second luciferase (Renilla). That's why I said all we've done is put this small viral frameshifting element between these two luciferase genes. We've just used 100 nucleotides of the SARS virus, and that's enough for us to study this frameshifting phenomenon.

GB: That's very interesting. Well, I'm now going to transition from you as a scientist to you as a person living through the pandemic. What have been some personal opportunities and challenges for you that COVID has presented?

TD: I have very personal challenges because I have an elderly mother living out of town, so that's been very challenging for my siblings and me in trying to take care of her. We removed all out-of-home caregivers for her (to reduce the risk of infection). While my sister has been doing almost all of the day-to-day caregiving, I've actually had to travel sometimes to help take care of my mother. Because I'm quarantining at home, I feel quite safe in helping. I'm not going out to the stores or anything, so I felt safe in helping take care of my mother. Fortunately, she just got her vaccine so we're very happy.

GB: Have you gotten vaccinated given that you are a practitioner at NIH?

TD: No, I have not. The people in my lab have been vaccinated. However, I have not been vaccinated because I'm working exclusively from home. I do not go to work at all because we have limits on the number of people allowed in the lab at one time. If I go to work, then someone who's doing experiments can't go to work (because of the occupancy limits). Because I really don't do the experiments anymore – I pretty much just sit at the desk and write – I am exclusively work from home (during the pandemic). The people in my lab who do COVID research have been vaccinated, but I'm still waiting for my name to be called, so that's a challenge. So, you asked about challenges, what was the other aspect?

GB: Opportunities.

TD: Opportunities. I'm not sure that it's opened up any new opportunities for us. We're still doing the same research that we've been doing so I wouldn't say it's provided… I mean other than what we're talking about here. We wouldn't be working on SARS if this had not been on the horizon (the need to respond to the pandemic had not arisen).

GB: Has it been difficult to or was it an adjustment to not be in the lab yourself and communicating with your staff by the computer or phone?

TD: Yeah, so I'm actually someone who likes to walk up to the bench and talk with the people in the lab as opposed to doing things by email, and so that's been a bit of a challenge. However, I zoom conference with everyone in the lab at least once a week and so we've kept that kind of face-to-face sort of communication, but, yeah, that is a challenge for me.

GB: Yeah. Well this is a fun question. What are you most looking for forward to doing as the weather gets warmer?

TD: During the pandemic, I bought an indoor bicycle so I'm really looking forward to getting outside to ride my bike. That's my biggest fun activity to look forward to … uh, yeah, I'm already thinking about it right now.

GB: So is there anything else that you would like to add as an NIH scientist but also as a person who is experiencing the pandemic like every other American right now?

TD: Yeah, so to me the main thing is perseverance. You know we have to be prudent in our activities, but persevere knowing that soon, even now, especially now, that we have the vaccines, we know soon things will get much better, but we have to persevere right now and maintain the precautions that we've been doing so that we don't botch it. That's what I keep telling all my family members, and we have to persevere for a few more months, and I think things are going to get a lot rosier.

GB: Yeah, well, thank you very much, and I wish the best for you in your lab, and I hope that you and your family continue to stay safe.

TD: Thank you.